Symposium on Membrane Biophysics

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EXCITABILITY PHENOMENA IN ARTIFICIAL MEMBRANES

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INTRODUCTION

The research of the origin of the "animal electricity" has a long history. We all know the fascinating story of the late 18th century Italian scientists Galvani and Volta's controversy about the role of electricity in the twitch of the isolated frog nerve muscle preparation. Nowadays, more than one and a half century later, we are still facing many of the problems, which baffled the early electrophysiologists. We still discuss the origin of the "demarcation" potential and the resting potential. Many theories were born and again gone with attempts to explain or, at least, to describe the most conspicuous bioelectrical phenomenon, the rhythmical or single action potential "spikes." All modern considerations of the nature of these events have one concept in common, they deal with transport processes of ions across "membranes." The problematics of modern electrophysiology is centered on the permeability properties and the structures of membranes and the nature of the driving forces transporting and assorting the main current carriers, the potassium and sodium ions.

The well known Hodgkin-Huxley ionic theory offers a quite successful quantitative formulation of various excitability phenomena of nerves or muscles in terms of what might be called a "two-force" system with specific permeability changes ascribed to the acting ions. The two superposed forces are the chemical potential and the electrical potential, the sodium and potassium permeabilities are defined as electrical conductances (dependent on the potential). This model has not yet found any inanimate analog system of a physicochemical nature. It is still an abstract mathematical conception adapted to the results of refined experimentation on biological objects.

If one approaches the phenomenology of electrical nerve actions from a more general biophysical viewpoint one soon becomes aware of the resemblance between the action potential display and various physical oscillation systems of nonlinear nature. Already Bethe characterized the heart beat potentials as "relaxation oscillations." Over the last decade numerous models of oscillatory, or unstable systems,

have been proposed, ranging from the well known iron wire model of Ostwald-Lillie to a host of electronic circuits, which mimic many of the essential features of the natural systems. Quite recently U. Franck (1) and R. FitzHugh (2) have given a generalized mathematical background, which includes many "excitable" systems, also the Hodgkin-Huxley formalism. It is obvious that the fundamental nerve actions, in spite of all its conspicuous uniqueness, is well adapted for a biophysical approach. However, as a characteristic of the majority of the proposed "nerve analogs" it may be said that they are either concepts, which are highly abstract, or composed of very few physiological "ingredients." We have therefore made an attempt to propose still another model, to be added to the array of all the previous ones. This might appear to be a daring enterprise, but the justification is, that we believe that the "model" or "analog" reproduces at least some of the properties of the life membranes. Accordingly in this lecture a "wet" physicochemical system will be described, which is designed to represent the cell conditions and exhibits "excitability" that is like many of the physiological events. We shall deal with electrolyte solutions separated by a boundary, the membrane, which includes a feature, which we consider to be important, namely the presence of immobile or *fixed ionic groups* in the porous matrix (= ionogenic, or shorter "ionic," membrane). Whatever view we might have of the submicroscopical architecture of the cell membrane, it seems difficult to escape the biochemical evidence that its proteinic and lipidic components must expose their own ionic groups (carboxylic, phosphoric acid etc.) within the membrane matrix, these groups necessarily being in a "fixed" state. Our system can be supplied with energy, and be stimulated, by an electric current. We shall also remark on the interesting fact that this analog as well is sensitive to pressure stimuli. Thus it may offer some viewpoints of another important physiological problem, namely the "mechano-electrical transduction."

PART I. A PHYSICOCHEMICAL MODEL (THE "MEMBRANE OSCILLATOR")

The "membrane oscillator" has been described in detail in several earlier papers, therefore only the main features of its design and the results need to be recapitulated here (3, 4, 5, 6, 16). The Fig. 1 shows its essential principles.

A thin porous, sintered glass (or porcelain) membrane separates two compartments with N/10 and N/100 NaCl. The membrane with its negative fixed charges arising from the presence of silicic acid corresponds obviously to the cell membrane and the left-hand compartment to the "inside" of a thought of cell, the right hand to the "outside". A constant D.C. current is flown across the membrane, creating a transmembrane potential being essentially an ohmic drop. The conspicuous phenomenon, which now occurs, which can be seen by the naked eye, are shifts of the levels of the free water surface. If the applied current is weak, a steady level difference is obtained after a little while, perhaps after some overshoot. This water-level differ-

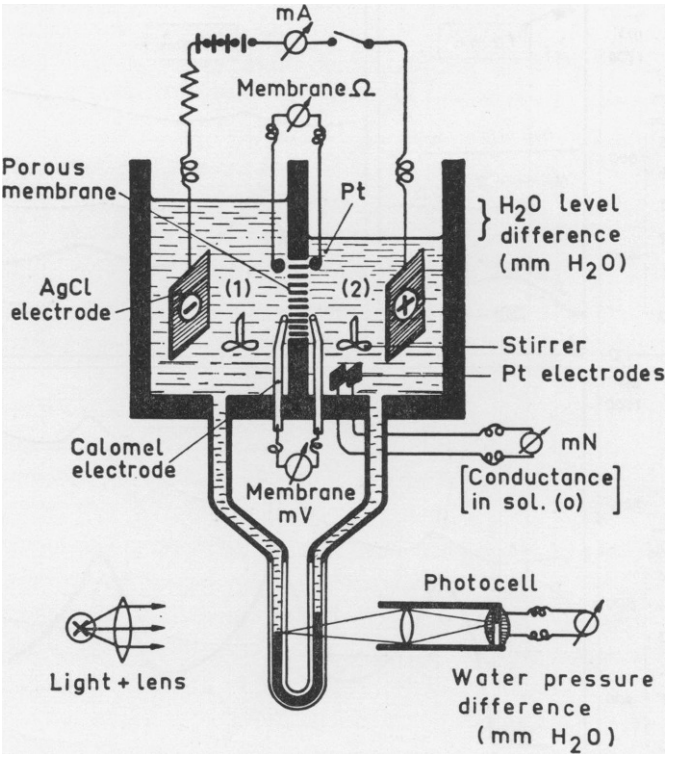

FIGURE 1 Scheme of the membrane oscillator (16).

ence corresponds actually to the *electroosmotic* pressure. However, if somewhat stronger currents were used, one may observe a cyclic or rhythmic variation like a damped oscillation. This might appear unexpected, but still more perplexing is the fact that a trifle higher current density suddenly sends the system into a continuous rhythmical oscillation of the water levels (Fig. 2). With appropriate electrodes, one can also record the transmembrane potential as well as the membrane resistance. Also these show a cyclical variation as shown in the figure. The potential variations can be said to be of the relaxation type and somewhat similar to an actual action potential train of a nerve discharge. We shall not here dwell on details of the experimental observations like those of Fig. 2. It may suffice to draw the attention to the fact that the level or pressure variations show a phase-lag in relation to the potential waves. This hints at a *coupled* system, in a very general fashion resembling the well known Volterra relations mentioned in Dr. Katchalsky's lecture at this meeting. So much for a general presentation of the behavior of the membrane oscillator.

Let us now turn to the discussion of *the biophysical characteristics* of this model. In comparison with the Hodgkin-Huxley model which operates with only two

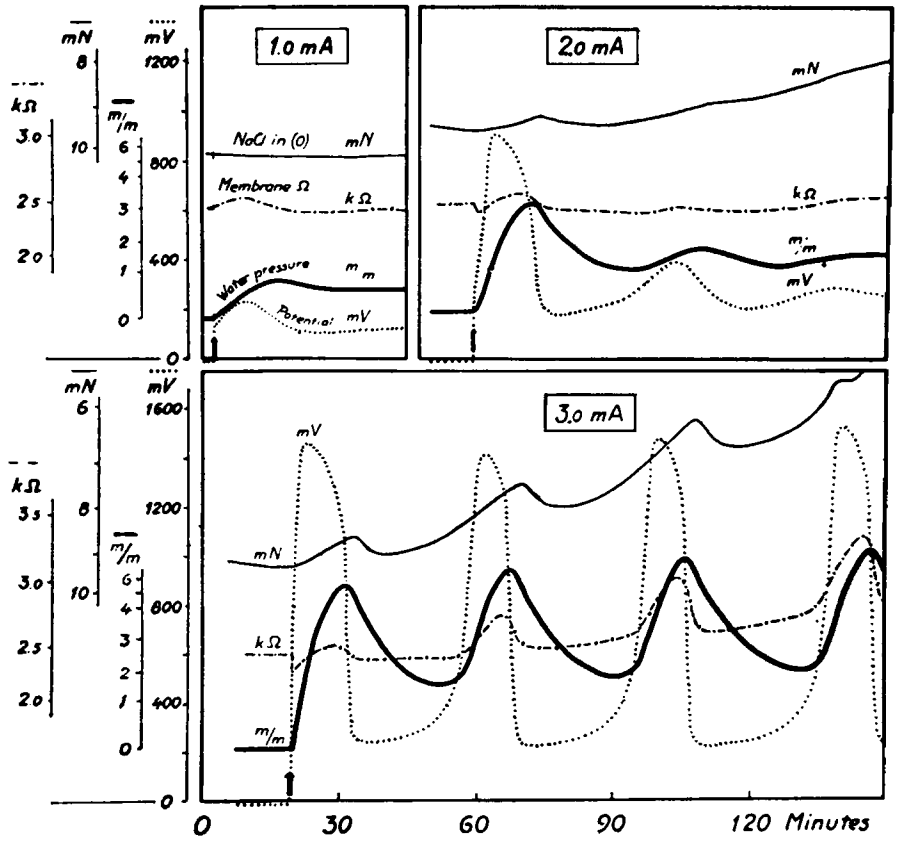

FIGURE 2 Experimental results with the membrane oscillator (16).

driving forces, the membrane oscillator is a "three force" system, where no specific permeability properties need to be considered. Furthermore, in contrast to the Hodgkin-Huxley system, the membrane transport processes include not only the mobile, dissolved ions but also the solvent, the water. In essence, the conception of the membrane oscillator—and its extension, the "electrohydraulic nerve analog," which will be presented in the later part of this lecture—is centered around electrochemical and electroosmotic transport, where the influence of the "fixed charges" of the membrane is especially considered. The transport of water gives rise to a hydrostatic pressure difference, so, in all, we deal with a) gradients of *concentration* (C) (chemical potential), b) gradients of *electrical* potential (E), and c) gradients of *pressure* (P). All these three gradients act in superposition adding up to a total driving force sum. Actually, it is this cooperation of "forces," which gives rise to the periodic or rhythmical "fluxes" of water and of solutes in the membrane oscillator. However, it is not easy to give a quick qualitative picture of the nature of these

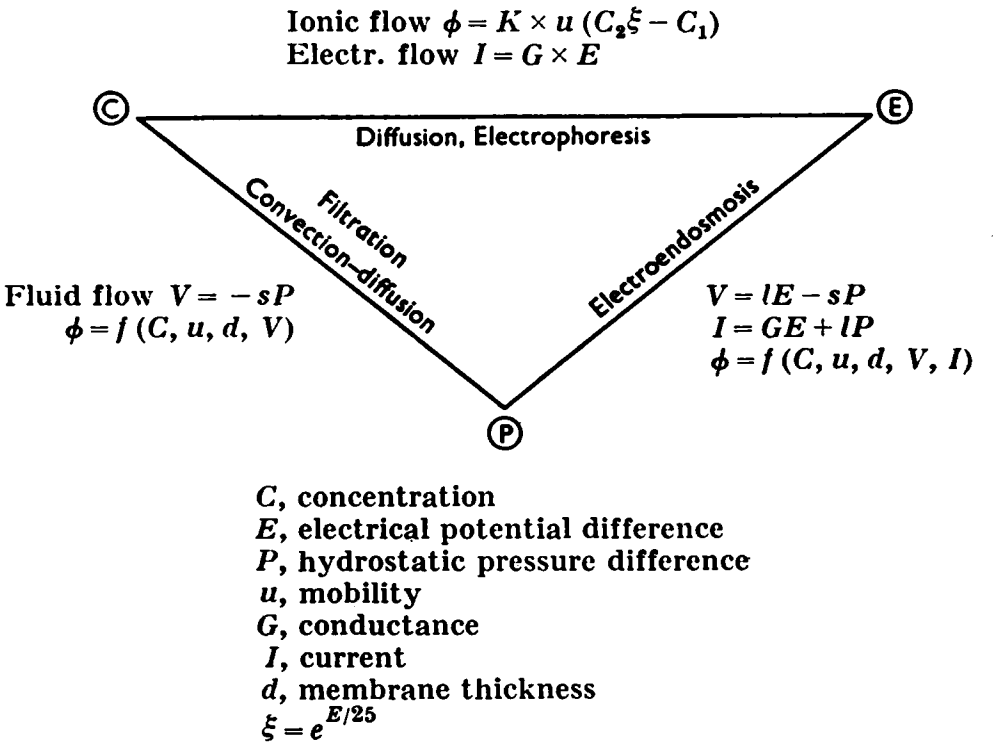

FIGURE 3 The phenomenological triangle (16).

events. We feel that a kind of survey of the most common transport phenomena may be helpful before continuing our discussion on the behavior of the membrane oscillator.

A classification of transport phenomena can be given pictorially as the "phenomenological triangle," Fig. 3. Here the three types of forces are placed in the corners and corresponding processes are somewhat schematically arranged along the sides of the triangle. It is hoped that the diagram is self-explanatory. The kinetics of transport is fairly well worked out for most of these processes, where the forces usually are taken in pairs. Attempts to deal with all the three forces are not so many. Some years ago R. Schlögl and U. Schödel (7) developed a new theory for so called "anomalous osmosis" in this fashion. Our contribution has been an attempt to describe the rhythmical processes under electrical current flow.

The core of the *analysis of the behavior of the membrane oscillator* is electroosmosis. We shall not here try to explain the intricate details of this phenomenon. It may suffice to say that it is a process bound to take place when one has a porous membrane with immobilized ionic groups attached to the wall of the pores, and it is driven by any electric potential gradient existing across the membrane. Somewhat

loosely speaking, the fixed anions in Fig. 4 would tend to move to the positive electrode through the water phase—if they could. Instead, however, the water layer close to the fixed charges will move in the opposite direction. The same *relative* movements of the fixed ions versus the solvent will thus be established as if "free" electrophoresis was present. The moving aqueous wall layer now exerts a drag on the adjacent layers, and, provided the pores are not too wide, it seems justifiable to assume a fairly constant flow velocity across a pore cross-section (so called "plug flow").

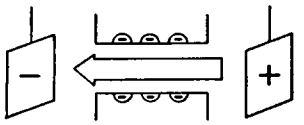

FIGURE 4 Scheme of electroosmosis.

The electroosmotic flow is a convection flow, which affects the concentration distribution within the membrane. If we make a kind of map of the concentration in different "slices" of the membrane, we obtain what has been called *concentration profiles*. It can be shown that the concentration profile in a membrane separating two solutions of different concentrations is a straight line, when no convection flow is present. On the other hand, a convection in either direction distorts the profile to a curve, which is convex or concave depending on the direction of the flow. Such profiles are exemplified in Fig. 5.

Now, however, different concentration profiles signify different total electrolyte content within the membrane. This means, in turn, that the electrical resistance across the membrane must depend on the direction and magnitude of the velocity, V, of the water flow across the membrane. We will get a high and a low resistance as limiting values at extreme V-s, because then the membrane will be filled with either of the surrounding solutions. At $V = 0$ the straight line profile will signify an intermediate electrolyte resistance. Accordingly, purely intuitively, it might be understood that the mathematical relation between resistance, R, and flow velocity, V has an S-shaped graph. Such a graph, mathematically calculated, is illustrated in Fig. 6. The reason why we emphasize this particular relation of $R^{\infty} = f(V)$ is that this yields a "backbone curve," having the same significance for the operation of the membrane oscillator as a vacuum tube characteristic has for the operation of a vacuum tube (although here we have to omit detailed justification for this statement).

We have also focused the attention on the concept of membrane "profiles" because they are of greatest importance in the *kinetical method* for treatments of more complicated membrane transport phenomena. This method of approach necessitates that one "looks inside" the membrane, to find out how concentration, or electrical potential, and pressure varies on the membrane inside. In contrast, we

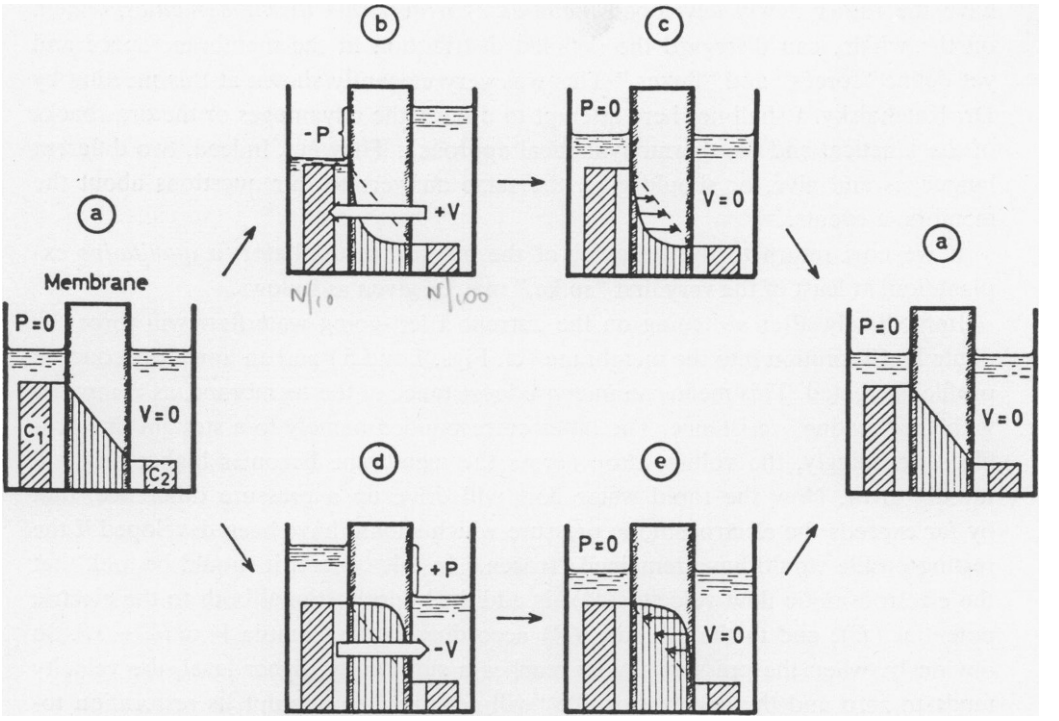

FIGURE 5 Concentration profile within a porous membrane as influenced by convection flow and diffusion (4).

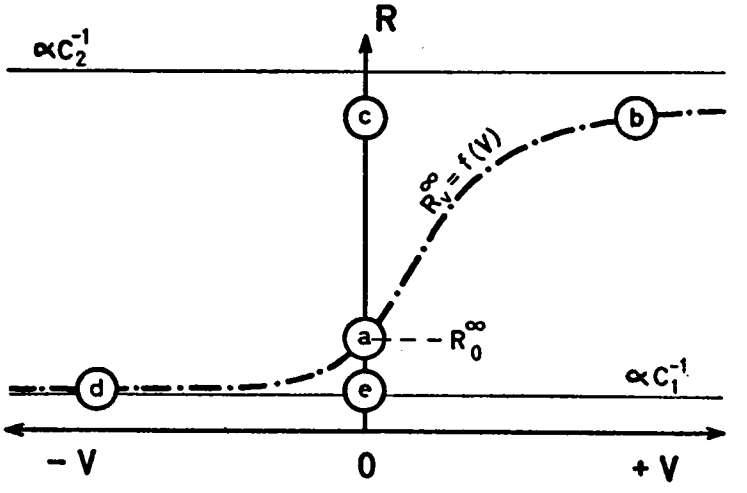

FIGURE 6 The relation between flow velocity, V, and steady state resistance, R^{∞} (4).

have the rather newly developed *methods of irreversible thermodynamics,* which, on the whole, can disregard the detailed distribution in the membrane space and yet define "forces" and "fluxes." This was very elegantly shown at this meeting by Dr. Katchalsky. I shall not here attempt to discuss the advantages or the drawbacks of the kinetical and the thermodynamical approach. They are, indeed, two different languages and give, or should give, the same answers to our questions about the membrane events.

If we now return to the behavior of the membrane oscillator, a *qualitative* explanation, at least of the very first "spike," may be given as follows.

Immediately after switching on the current a left-going waterflow will force the dilute NaCl solution into the membrane (cf. Figs. 1 and 5) and an upwardly concave profile is created. This means an *increased* resistance of the membrane, as compared with the "resting" resistance. The latter corresponded namely to a straight line profile. Accordingly, the voltage drop across the membrane becomes higher and will rapidly grow. Now the rapid water flow will drive up a pressure difference, that by far exceeds the electroosmotic pressure which should have been developed if the resting profile would have remained "frozen." In this context it should be told, that the electroosmotic flow velocity (V), is additively proportional both to the electric potential (E), and to the pressure (P) according to the formula $V = lE - sP$. So obviously, when the pressure finally reaches a sufficiently higher level, the velocity tends to zero and the concave profile "will get time" to attempt its restoration towards the steady state straight line. This is a comparatively slow process because it is governed by a diffusion process. At this instance, however, there is an excess pressure difference above the electroosmotic pressure corresponding to that steady state (because the potential has been "too high" during the first time). Hence, a water flow will start in the opposite direction, which flips the profile more and more to a convex shape with a simultaneous *decrease* in resistance and potential. Thus, we have followed a rise and a decline—a part of an oscillation. I hope that this follow up of the initial events will be an aid for the understanding of the oscillatory machinery. Summarizing, one may perhaps say, that the water flow acts as a *distortive* factor on the concentration profiles, while the slow diffusion is a *restorative* factor—and these two chase one another continuously, if sufficient energy is supplied for maintaining oscillations (= sufficient current density).

The hints given as to the qualitative events within the membrane give admittedly a somewhat poor picture. After all, it is more rational to put together *the governing mathematical equations* and see what they lead to. This quantitative analysis has been given in some earlier papers (4, 5, 6). It was found that the membrane oscillator is governed by five relatively simple equations, which act simultaneously. The five equations run as follows:

$$dP/dt = (1/q) \cdot V \qquad \text{Eq. 1}$$

$$V = lE - sP \qquad \text{Eq. 2}$$

$$i = E/R \qquad \text{Eq. 3}$$

$$R^{\infty} = f(V) \qquad \text{Eq. 4}$$

$$dR/dt = k(R^{\infty} - R) \qquad \text{Eq. 5}$$

A few *comments on the meaning* of these equations may be appropriate.

Eq. 1 is simple enough, it just states that the rate of change of pressure (P) is proportional to the water flow velocity (V).

Eq. 2 is the classical law for electroosmotic velocity, being additively composed by a potential (E) and a counteracting hydrostatic pressure (P): l is the electroosmotic permeability coefficient (being proportional to the fixed charge density of the ionic membrane): s is the hydraulic permeability constant of the membrane (the "porosity").

Eq. 3, being Ohm's law, ought actually to contain a "cross-term" lP in the right member. Thus, it could be paired with Eq. 2 and could be discussed in terms of the Onsager relation as required by irreversible thermodynamics, (V is the "flow" of the solvent, i is the "flow" of the ions).

Eq. 4 is nothing but the function already graphically depicted in Fig. 6, called the "backbone curve" showing the relation between the steady state resistance and the water velocity.

Eq. 5, finally, expresses the assumption, that the rate of change of the instantaneous resistance (R) is put proportional to the divergence from the corresponding steady state resistance (R^{∞}).

It can be shown, that the five fundamental equations above are readily recombined to a set of two non-linear differential equations. These are somewhat bulky and should be omitted here. However, when they are solved they yield damped or undamped oscillations of the "state variables" R, E, P and V. The algebraic solution procedure is a graphical one and is rather cumbersome and time-consuming. Fig. 7 may give some idea of such a graphical prediction of the behavior of the membrane oscillator. A solution with an analog computer machine is far more convenient and flexible. In Fig. 8 is shown a simplified analog computing program, which also is an operational scheme of the functional interrelations described by the five fundamental equations. It is observed, that the diagram depicts a closed loop system with feedbacks. From this it follows, that a perturbation (= "stimulus") of any of the parameters within the loops will cause a propagation of a corresponding (transient or oscillatory) disturbance (= "response") throughout the system.

In the particular case of the figure the perturbation is a constant forcing by the current i. If we now follow this "flow chart" around the loops we find the following. The current is multiplied by the resistance (R) thus creating the potential (E). The potential, in turn, operates on the velocity (V) by virtue of a electroosmotic permeability coefficient (l) (being proportional to the fixed charge density). V, in turn, contributes to the pressure (P) which feeds back on the V again, thus fulfilling eq.

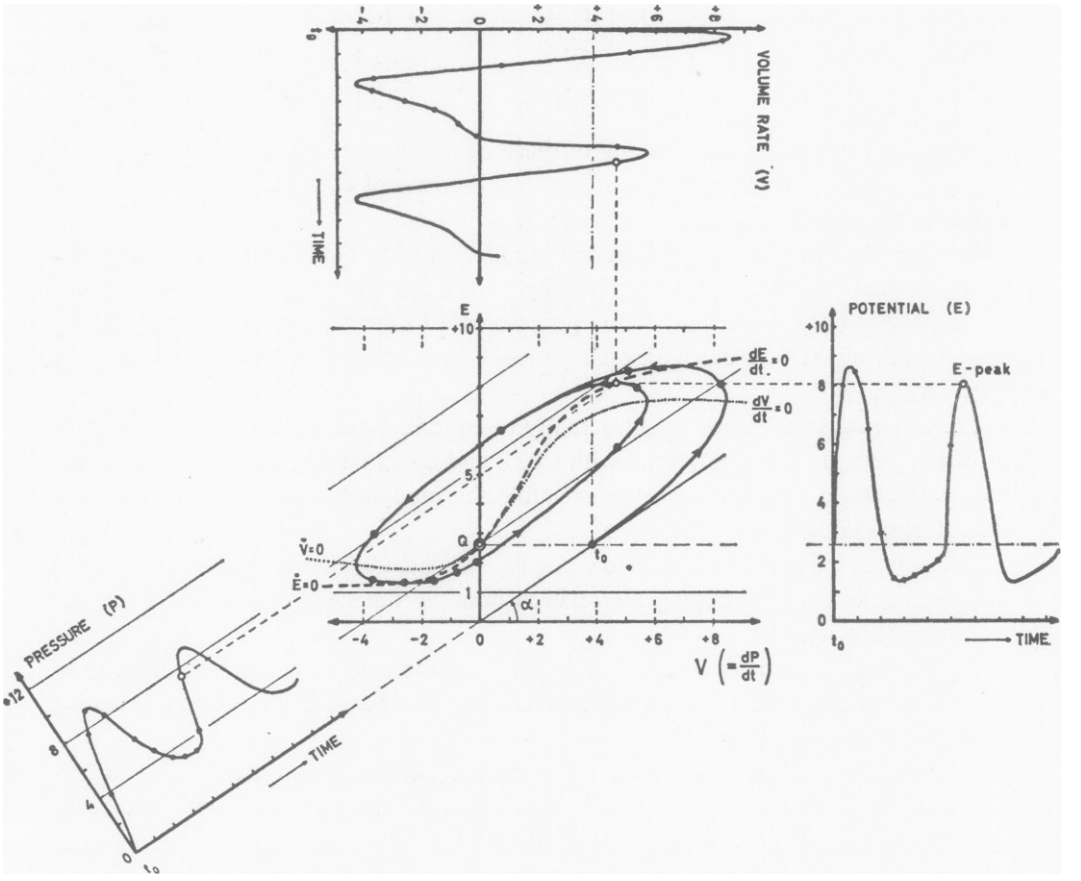

FIGURE 7 A graphical solution of the membrane oscillator equations (4).

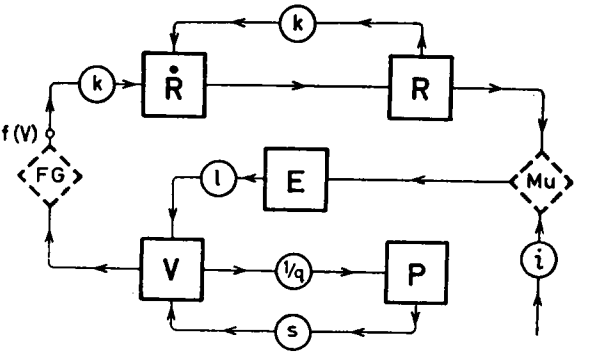

FIGURE 8 Operational scheme of the membrane oscillator (4).

(2). The velocity also influences the resistance behavior (according to Eqs. 4 and 5) via $f(V)$ and $\dot{R}$ and arriving back to R, we have travelled around the loops.

The actual operation of the analog computer gives results, which quite faithfully reproduce the actual results of the membrane oscillator. The analog procedure has also the additional advantage, that it is easy to examine—just by turning knobs of potentiometers—how changes of the parameters, for instance the fixed charge (l) or of the hydraulic permeability (s) influences the behavior of the system.

We hope, that the description of the results and the theory of this physical model, fragmentary as it is, yet has given a general idea of its functions and features. It is obvious, that the real model also allows chemical analytical flux studies and the use of isotopes for elucidation of the ionic transport processes. We have performed such studies and the results fit rather satisfactorily with the time course of fluxes simulated on the analog computer (unpublished material).

Some remarks on gel membranes. In the work with rigid membranes with a relatively weak charge density (glass membranes, porcelain membranes), as described hitherto, it was always necessary to maintain a rather marked concentration difference between "inside" and "outside" solutions of the order of 10:1. This is not a common physiological condition, that the extracellular liquid is more dilute than the intracellular. However, we have recently found that rather loose membranes of a higher charge density may exhibit oscillations also when the inside and outside are of equal concentrations (8). In these experiments the passage of a constant current induces a skew concentration distribution *within* the membrane and we obtained thus the same conditions as in the earlier experiment with initially unequal outside concentrations. Fig. 9 illustrates such oscillations, which, we think, may come somewhat closer to actual physiological conditions. When dealing with this new type of membranes elastic and swelling properties introduce new sets of interesting parameters (cf. Fig. 18).

PART II. THE ELECTROHYDRAULIC EXCITABILITY ANALOG

Part I has dealt with actual physicochemical events in a real ionic membrane (= fixed charge membrane), which was porous enough to allow water to pass. We imagine that such a membrane to some extent reproduces the physical and biochemical features of living membranes. From what we know from their biochemistry they contain proteins, lipids and other substances, which must contribute quite a high volume density of *fixed ionic* groups, like carboxyl groups, phosphoric acid residues, possibly amino groups etc. One can certainly discuss what degree of "porosity" the living membranes might have, as well as the architecture of its charge distribution. Many modern authors, for instance of the Solomon school, have determined "effective" pore diameters of the order of 5–10 Å. This is far less than the pore width in the membrane oscillator described above, which was of the order

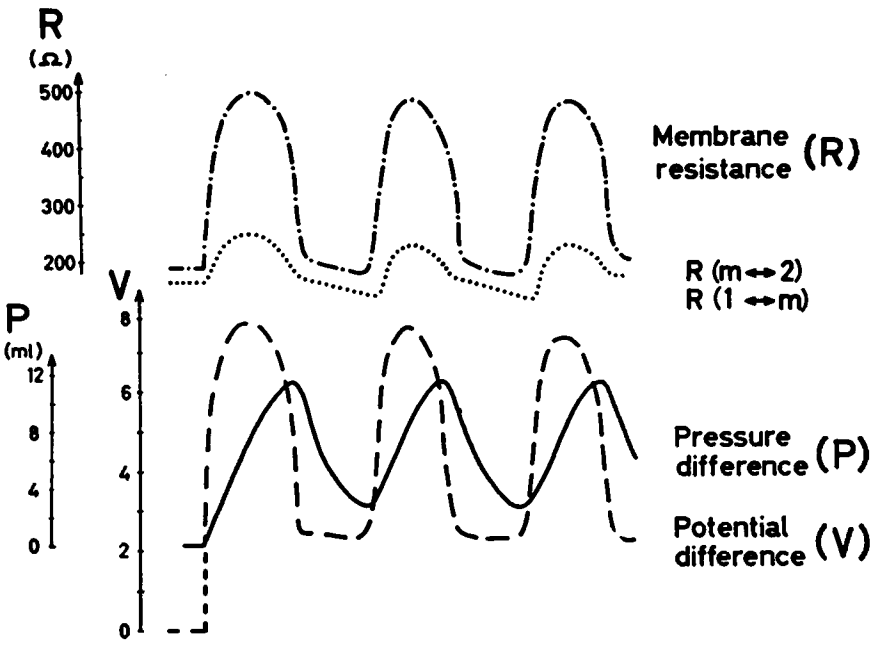

FIGURE 9 Oscillatory behavior of a negative gel ionic membrane with equal surrounding solutions (8).

of 0.1 μ or more. However, we do not know for certain down to what dimensions electroosmotic phenomena occur. As regards the charge of the pores, it seems perhaps unrealistic to reproduce this with only one pore category (of a constant fixed charge density), as we did in the theories outlined in Part I. One might also conceive of both negative and positive membrane channels side by side or even in series.

Apparently we know very little for sure about the facts of pores, their dimensions and their charge architecture. In such a dilemma we have chosen to represent a biological cell membrane system somewhat arbitrarily as illustrated in Fig. 10. We have still maintained the idea of fixed charged pores (the "electrical pores") but also introduced uncharged pores in parallel. These "leaky pores" do convey water streaming only when subject to a pressure gradient. This new system is comparatively easy to reproduce both in actual models and, in particular, on the analog computer. We shall, in what follows, entirely deal with the mathematical system, as operated by machine computation. The functional scheme of Fig. 11 illustrates, that the only modification of that of the membrane oscillator proper (cf. Fig. 8) is a new feedback path between pressure and velocity (V_L). The success of this model as regards the simulation of actual biological excitation phenomena is such, that we have ventured to call this modification of the membrane oscillator with a rather pretentious name, the "electrohydraulic nerve analog."

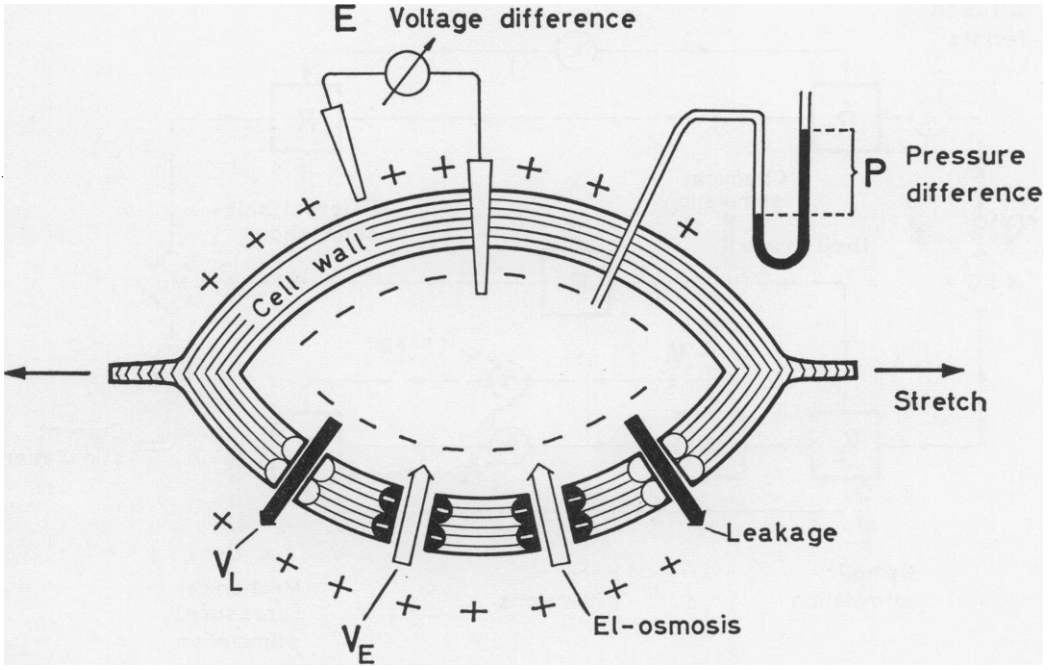

FIGURE 10 Schematical representation of the electrohydraulic excitability analog (5).

A list of the excitability phenomena, which we have treated, is shown in Table I. Admittedly, this list is a somewhat unlogical condensation of many different phenomena, described within the field of neurophysiology. Also we have to take for granted that its special nomenclature is known to the reader. It is not our intention, of course, to go through all the various points of the table at this occasion. We will select only a few in order to illustrate what can be obtained from this analog.

1. *Demonstration of some excitability phenomena. Threshold, subthreshold response* and *all-or-none response* are depicted in Fig. 12a) for both *cathodal* and *anodal* current stimuli. In the top section, the transition from a small subthreshold response to a full-sized action potential is caused by few per cents in stimulation strength, not distinguishable in the record. Also the "afterhyperpolarisation" is very similar to that sometimes displayed by living nerves. (The "overshoot" observed is due to the fact, that the total potential is given as $E = IR + e$, where IR is the ohmic voltage drop and e is an opposite constant potential assumed to be present here, compare the scheme of Fig. 11). The difference in potential response between two identical cathodal and anodal stimuli are seen in the middle section. In the lowest section the latency differences of varied anodal (break) stimulations can be seen.

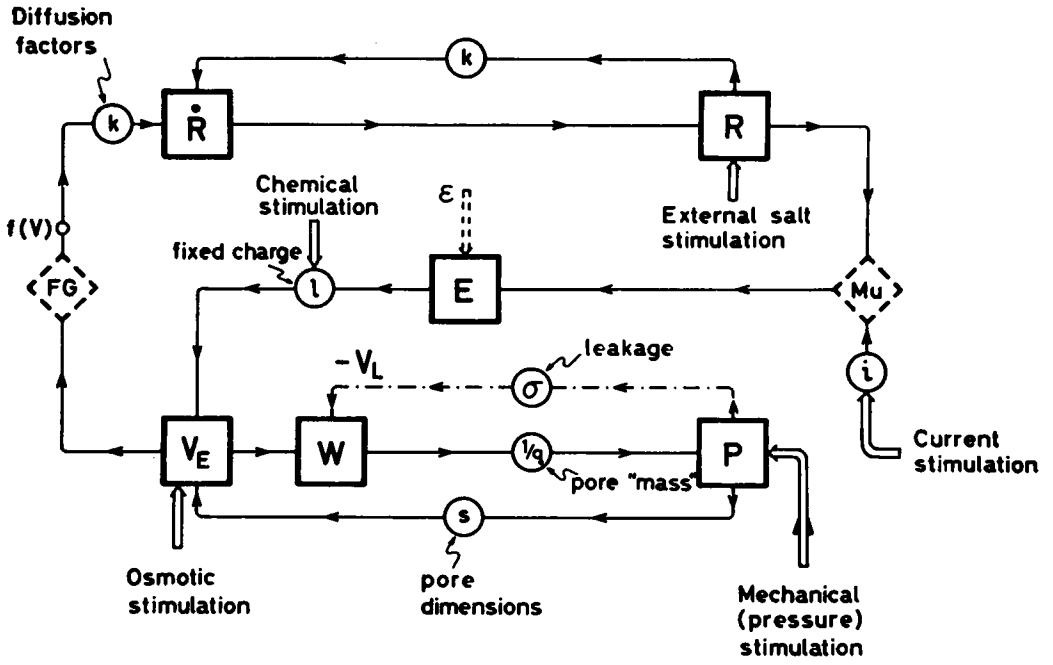

FIGURE 11 Operational scheme of the electrohydraulic analog showing also various modes of stimulation (5).

Cathodal and anodal stimulations are known to cause so called *"break"* and *"make"* stimulations respectively. This can also be observed in Fig. 12a.

Refractoriness is demonstrated in Fig. 12b) where identical stimuli are given right after a spike at three, slightly different time intervals. One may easily distinguish between an "absolute refractoriness" in the top section and an abortive response in the middle section indicating "relative refractoriness," and something like a doublebeat in the lower section, all resembling what has been observed on physiological objects. A related effect is *"abolition"* where anodal pulses instead are imposed on the spikes and, indeed, the characteristics of abolitions are faithfully reproduced by the computer although examples will be omitted here.

The strength-duration relation is known in excitation physiology to be of a hyperbolic shape and are wellknown from so called chronaxy studies. In Fig. 12c) are shown a cathodal, respectively anodal, *S-D* relation as obtained on the present analog. Again it is noticable that the anodal stimuli have less effects. The hyperbolic nature is also obvious.

The so called *"frequency modulation"* is the information code of the nervous system. It is the number of spikes per time interval, which conveys the nerve message, not the amplitude (which ordinarily is approximately the same). The frequency modulation is such in living objects that a stronger stimulus usually produces

TABLE I

Single action potential (A.P.)

Initiation (triggering): Threshold, subliminal activity, all-or-none A.P.
Cathodal—(make); Anodal—(break) response
Strength-duration relation.

Repetitive A.P. (Rhythmicity)

"Frequency modulation"
Refractoriness ("extrasystole")
Abolition
Accommodation, adaptation, fatigue

Special trigger modes:

Single pulse trigger
Single pulse "annihilation"

Special phenomena.

Rectification, "bistability," *E-I* characteristic
Impedance change
High KCl—anodal repolarisation
Voltage clamp procedure
Square wave-& triangle wave analysis
Ion fluxes.

Non-electric stimuli.

Pressure differential (Mechanical)
Osmotic
Chemical.

a more high-frequent response train. The Hodgkin-Huxley theory predicts a moderate range of possible frequencies (as analysed recently by FitzHugh (9)). This is also the case, but within a more narrow limit, with our analog system as we ordinarily use it. However, if one introduces the assumption, also used by others (10), that some electrolyte can accumulate temporarily on the outside boundary of the membrane, before it is removed by convection and diffusion, it is possible also on the present analog to obtain a quite wide range of response frequencies. This is seen in Fig. 13 where three slightly different depolarisation levels were maintained. Regrettably the time does not permit to enter upon how the ion flux equations are handled on the computer. Also "fatigue" may be provisionally dealt with, in a related manner.

The mechano-electrical transduction i.e. the elicitation of an electrical signal by a mechanical (pressure) stimulus has for a long time been a challenge to the sensory physiologists. We know of systems, which have an extreme sensitivity to pressure, for instance the hearing apparatus. Furthermore there are the presso-receptors in the skin, the tendons etc., which react to deformation or stretch. How is this transduction phenomenon accomplished? Very few theories have been offered in terms

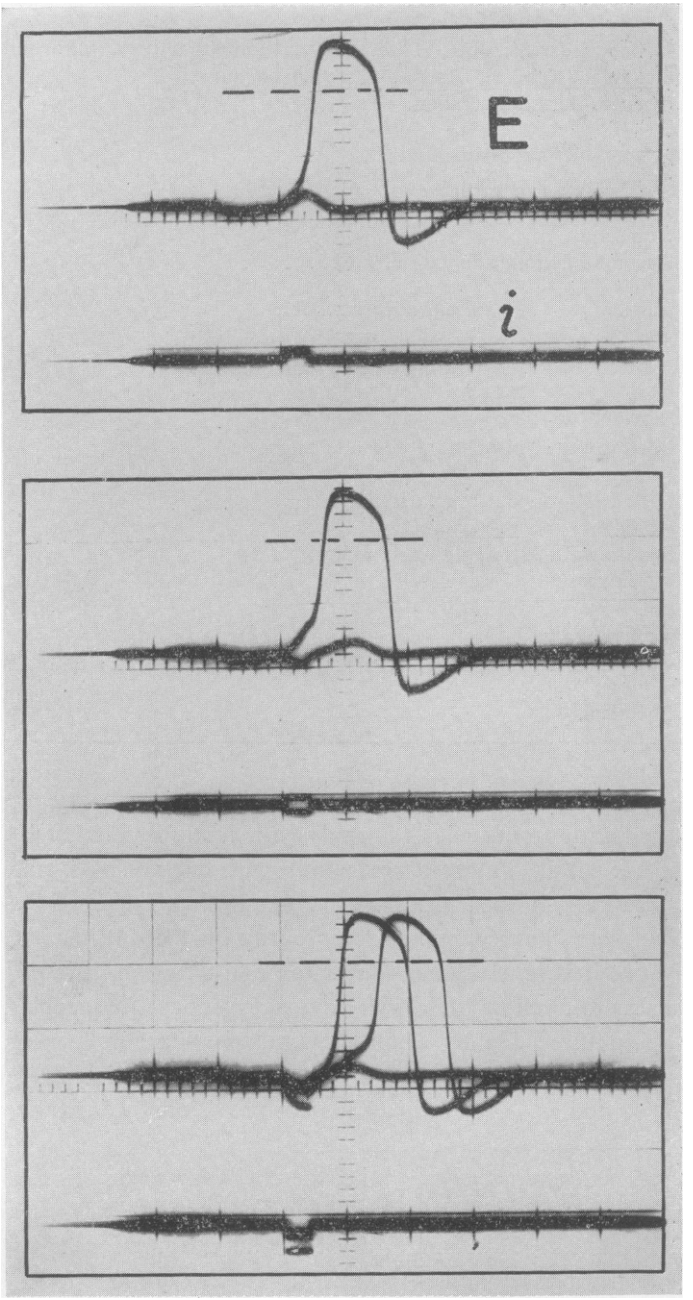

FIGURE 12 a Various types of responses with the electrohydraulic analog. Cathodal and anodal responses.

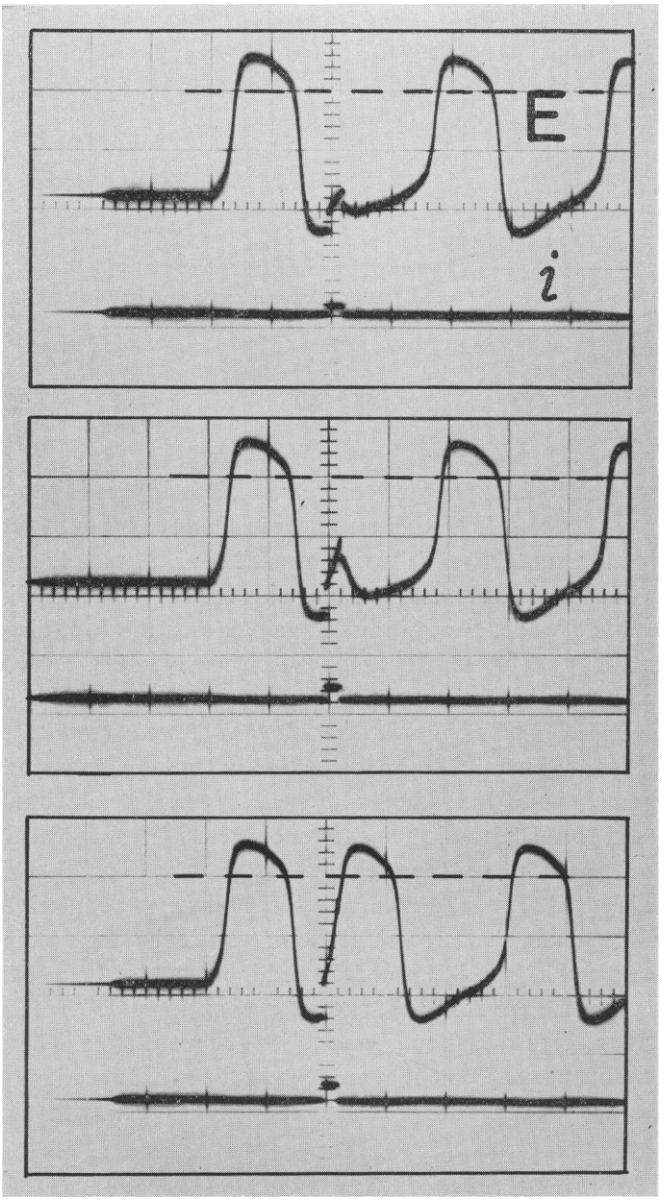

FIGURE 12 b Various types of responses with the electrohydraulic analog. Refractoriness.

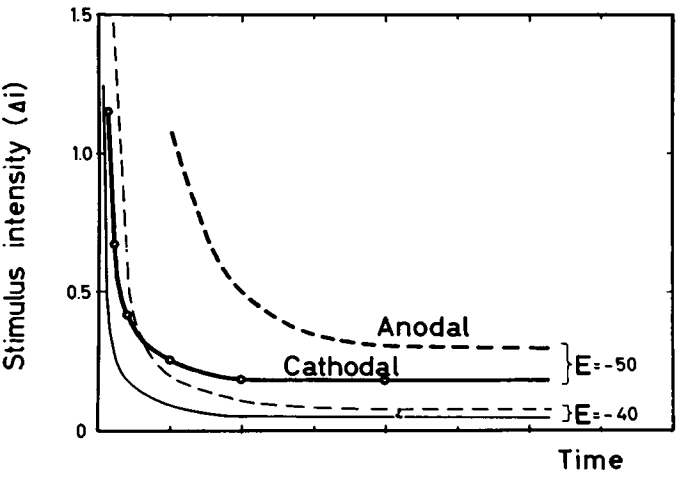

FIGURE 12 c Various types of responses with the electrohydraulic analog. Strength-duration relation curves.

of physiological possibilities. In view of this fact it is interesting to note, that the present analog is capable of eliciting electrical instability and even action potentials following a pressure stimulus (this was shown also for the simple membrane oscillator (3)). In Fig. 14 is demonstrated that also for pressure stimuli there exists a very sharp threshold value. Here we have again chosen the R-V-diagram, which is quite useful in graphical presentations of the electrohydraulic analog. One should note that the pressure axis has an oblique course in relation to the other two axes.

2. *The membrane events in the electrohydraulic analog.* After having demonstrated some "simulation" of nerve action potential phenomena we will again somewhat closer analyse what actually happens in the electrohydraulic system. As before it is useful to relate the intramembrane electrical resistance, R, to the water streaming velocity, V. Such a representation is given in Fig. 15. Here the resting state is denoted by "Q-state." At a current stimulation (= depolarisation), marked S, there is a sudden change (decrease) of the water velocity, the system is thrown out of its steady state and a "path" is now followed indicated by the arrow heads. There is first a decrease in the resistance, R, and after a considerable "impedance loss" the path returns, after some overshoot, back to its quiescent state. The corresponding excursions in the membrane potential are seen in the middle section of Fig. 15. The translation into the time-variation is also shown, together with the concomitant change of the pressure, P.

Again we wish to draw attention to the fact, that all these events, in essence, involve shifts or deformations of the concentration profiles (as shown for the active (A) and quiescent state (Q) in the lower lefthand section of the figure). Of special interest may be the fact that there is an outward bulk flow at the spike of the action

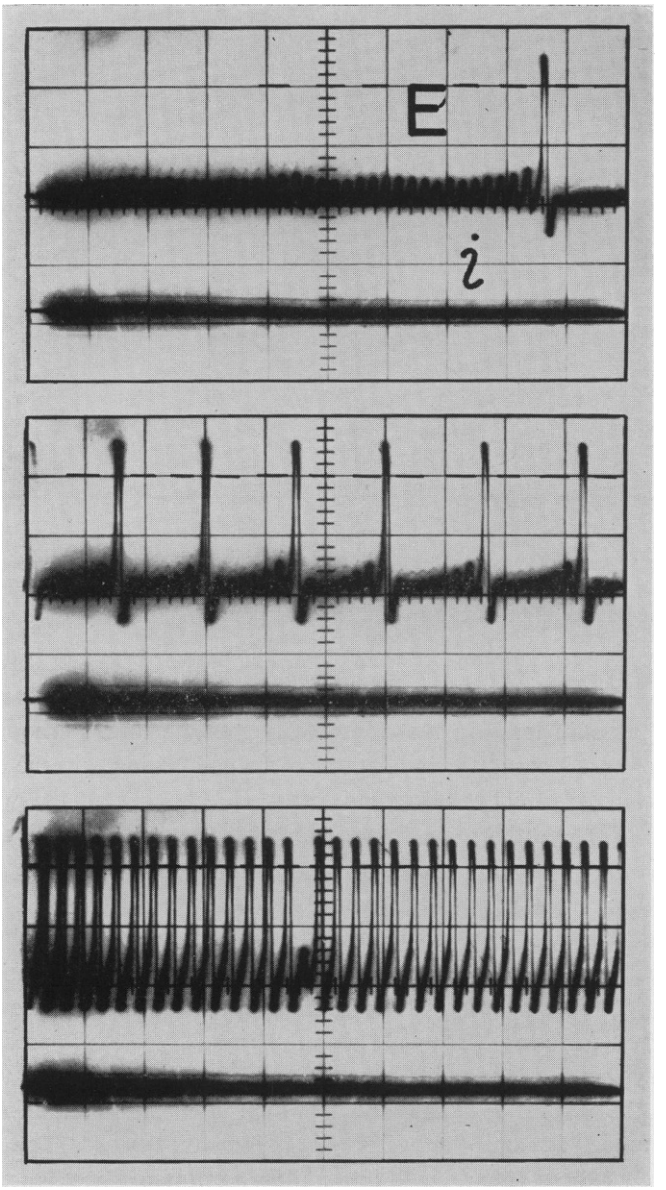

FIGURE 13 "Frequency modulation" at various depolarisation levels.

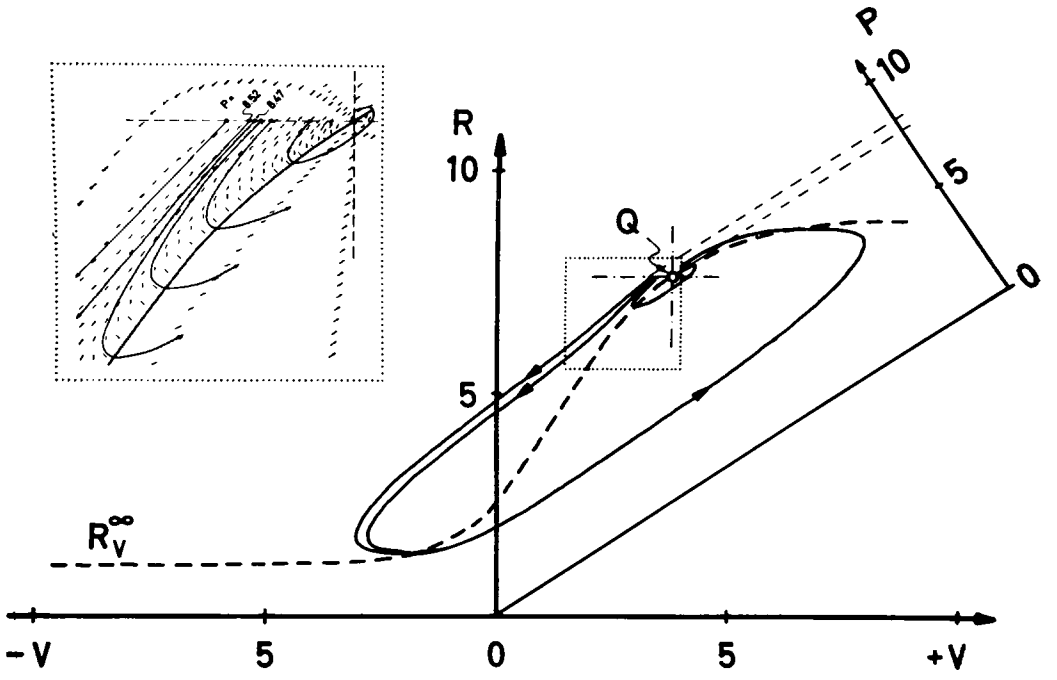

FIGURE 14 Elicitation of action potential response by pressure stimuli. In the inset note the sharp threshold that causes a fullsized response.

potential. In other words, in this particular "nerve" one would expect a "volume loss" and a "pressure loss" in the excitable membrane unit. Perhaps it would be worth while to start looking for indications of volume changes in the actual nerves. Some attempts have already been made to study changes of the water state by Julian Tobias (11) and there are some interesting reports by Russian investigators (12, 13), which might be interpreted as small volume changes on the nerve surface, concomitant with the action potential trains.

As model-makers we have now created a rather complicated piece of "nerve research," because we have been forced to study not only the conventional parameters, the electrical potential and the resistance, but—and this is a new aspect—we have been forced to follow also water streaming and hydrostatic pressure changes. Indeed, our system now contains a great number of interrelated variables: the resistance, R, the current, I, the potential (which is, in the main, an IR-drop), the pressure difference, P, and the water streaming velocity, V. The simultaneous presentation of all these would result in a multi-coordinate space figure and be quite formidable. By using oblique coordinates some presentation can be made as we have shown in the figures 7 and 14. Out of many possibilities for interrelating the "state variables" we will now concentrate on the following, namely:

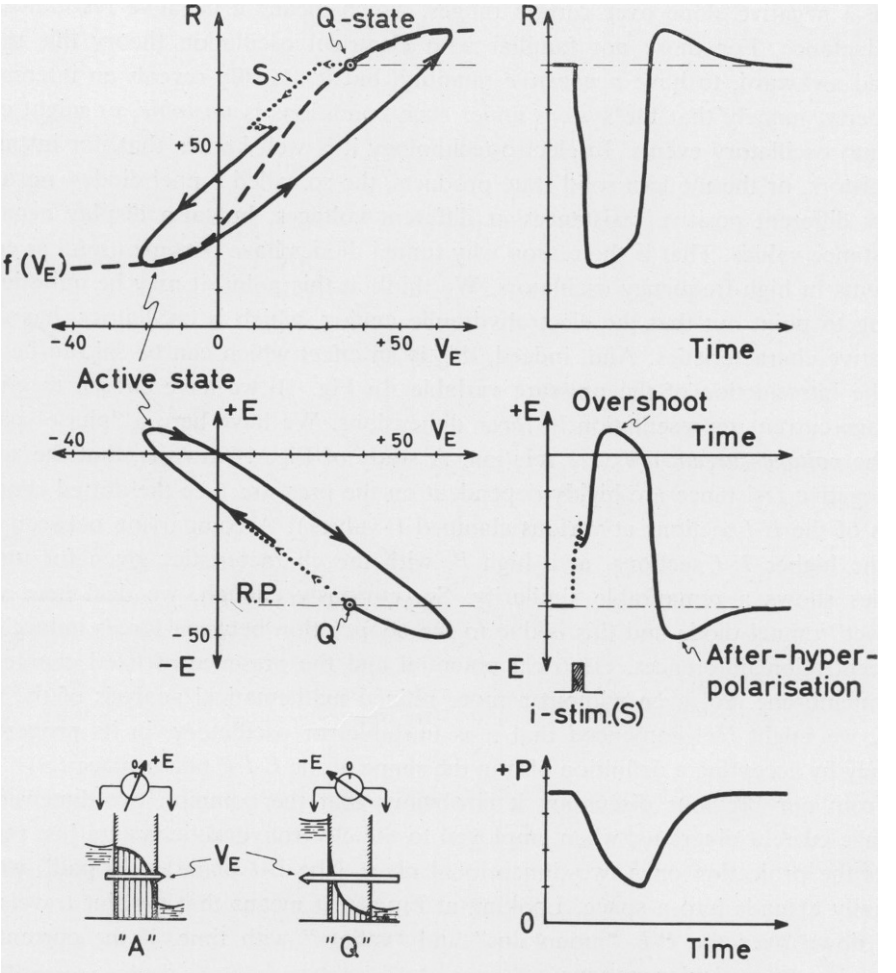

FIGURE 15 Diagram of the events during an action potential (E).

3. *Voltage-current relations.* Nowadays a common method of characterising the behavior of excitable tissues is in form of a diagram of two "state variables," the stimulating current, I, and the resulting potential, E. Such "voltage-current representations" are very useful and can be found abundantly in the literature. Corresponding E-I curves can be easily worked out for the electrohydraulic system. The resulting curves can, again, very closely mimic many of those described for living material. In connection with the popular "voltage clamp procedure" the E-I diagrams are often used. In passing, we may mention that the electrohydraulic analog also shows characteristic *voltage clamp* responses, although we should not embark on this at this occasion (see ref. 6 for further information).

In many of the published voltage-current graphs one can often notice they

have a negative slope over certain ranges, which means a negative resistance, or conductance. For those not familiar with electrical oscillation theory this might sound awkward, to have *a negative* quantity, but it actually reveals an interesting property, namely that the system under such conditions is *unstable,* or might even go into oscillatory events. In electro-technology it is well known that, for instance, termistors, or the modern solid state products, the so called tunnel diodes, not only show different positive resistances at different voltages, but also display negative resistance values. That is the reason why tunnel diodes have become useful as components in high-frequency oscillators. We think at this point, it may be quite interesting to point out that the electrohydraulic analog, which is oscillatory, has such negative characteristics. And, indeed, this is an effect which can be said to be due to the introduction of the pressure variable. In Fig. 16 we have chosen to give a voltage-current representation in *three* dimensions. We have here a "phase space" of the *voltage-current-pressure* relation. A study of Fig. 16 reveals, that the zones of negative resistance are highly dependent on the pressure (see the dotted contour lines of the *E-I* sections at various clamped *P*-values). A comparison between any of the higher *E-I* sections, at a high *P*, with the characteristics given for tunnel diodes shows a remarkable similarity. So, curiously enough, we deal here with a "wet" tunnel diode and this is due to the cooperation between forces induced by concentration differences, electrical potential and the presence of fixed charges in the membrane. So, even without a more refined mathematical analysis of the system, we might feel convinced that it is unstable, or oscillatory, in its properties, merely by accepting a definition of it in the shape of the *E-I-P* phase space (8).

From our previous discussion it is obvious that the common two-dimensional voltage-current diagrams, when employed to the electrohydraulic system just represents the projection on a two-dimensional plane (the *E-I* plane) of a path, which actually extends into a space. Looking at Fig. 16 it means that a point travels up and down over the *P-E* "mountains" and "valleys" with time, if the current, *I*, varies in any particular manner.

To give an illustration to this *E-I-P* philosophy, we will cite an example of an experiment carried out on a real living cell, the Nitella algae (15). This was depolarised and repolarised with a symmetrical, "triangular" current wave of constant amplitude. By means of an X-Y-plotter Fig. 17a was obtained as a conventional voltage-current relation. In this particular case one got a "loop," because we were interested in the "dynamic" characteristic and, hence, used a rather rapid current variation. The conspicuous feature of this loop is the quite sudden "flip-over" of the potential at a certain current value. A minor such is also seen on the repolarisation part of the hysteresis like loop. Obviously these flipflops represent *unstable states* of the Nitella. For comparison it is interesting to look upon Fig. 17b, which is a "triangle wave experiment" simulated on the analog computer. It should be admitted, that some adjustments of the parameters had been made, but the system

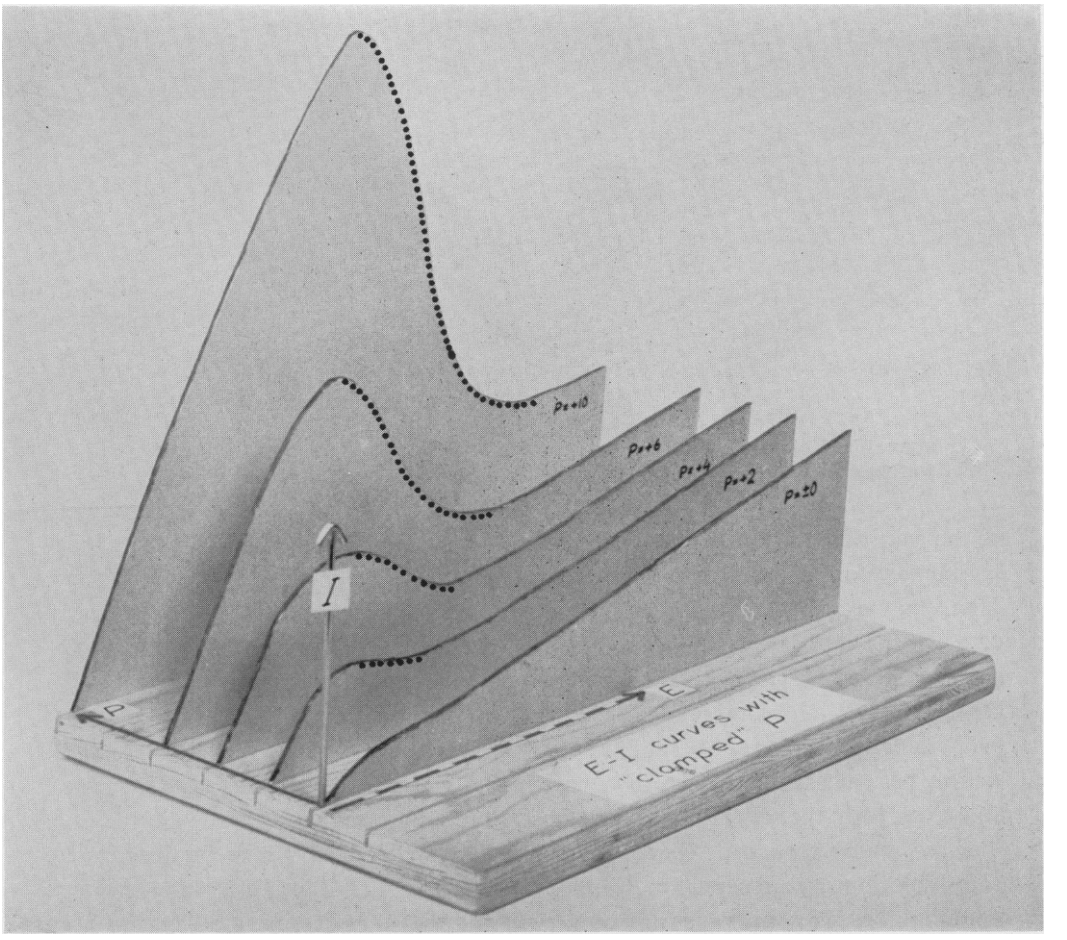

FIGURE 16 The phase space diagram of the relations between the three state variables of the electrohydraulic analog. Note the dotted contours indicating negative conductance. (8).

was essentially the same as that which was given in our previous presentation. If we disregard the fact, that there is always a finite current present (a "resting current"), there is a remarkable similarity between the artificial hysteresis loop and that of the Nitella cell. The resemblance between the life and the artificial curves is so striking that one might feel tempted to believe, that the Nitella possesses a third state variable, possibly the pressure, in addition to the current and voltage.

It should be pointed out, however, although we feel that the hydrostatic pressure would be a likely third state variable, that Nature may have employed something else. A system with coupled variation of other state variables is, in essence, the background of a recent fine presentation by FitzHugh, who deals with the application

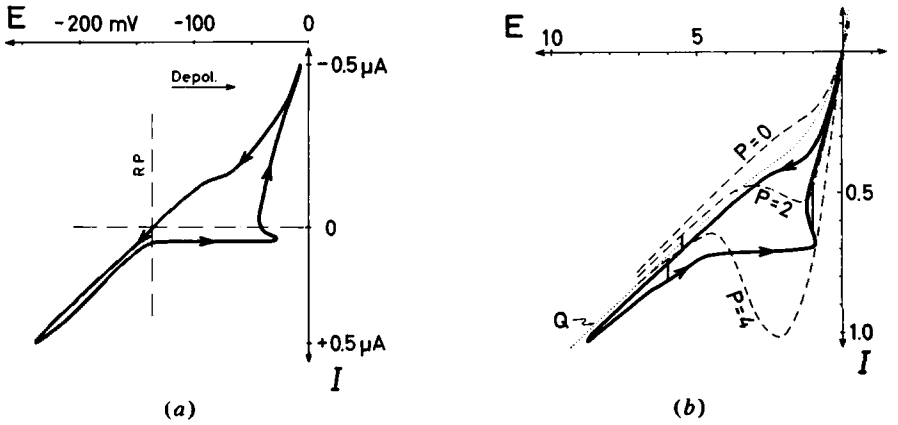

FIGURE 17 Voltage-current diagram. *a*) "Triangle wave" response of a *Nitella* cell. —*b*) Corresponding experiment on the electrohydraulic analog. (15).

of the van der Pol equation—well known from electrotechnology—to the Hodgkin-Huxley formalism. These considerations emphasise a menace for modelmakers. There may exist many systems, that may be quite different in physical, or chemical nature, and yet, they can have a common mathematical description. From a biophysical point of view there is seldom anything unique and convincing about a certain model—and although our pictures and our presentation has been somewhat deliberately shaped to show similarities with the actual behavior of excitable tissues, we are fully aware of the fact, that there may exist many alternative model theories, able to perform the same duty as the "electrohydraulic" analog.

Some remarks on the "configuration" of the excitable unit may be appropriate before closing our presentation. So far, we have assumed that the ionic membrane, imitating the cell membrane, has been *rigid* and that the pressure gradient has been located all across the membrane, with the "inside" of the "cell" being at a higher pressure or "turgor." This was the assumption for the scheme of the electrohydraulic model as represented by Fig. 10. However, it is not likely that the biological membranes are rigid, they may rather be distendable and (visco-) elastic. Thus, reaction forces, balancing a possible electroosmotic pressure, may be developed *within* the membrane structure itself. Furthermore, different layers in the composite membranes may have varying charge densities and hydraulic permeabilities. Accordingly, it does not appear too far fetched to suggest another configuration of an electrohydraulic membrane system, such as Fig. 18. Here, the whole excitable unit is confined within the membrane layer. We have not yet any experimental evidence as to whether such an artificial system would work rhythmically. We know from the recent work by Baker, Hodgkin and Shaw (14), that the excitable machinery seems to be located in the nerve axon "skin," and is not necessarily dependent on the presence of an intact axoplasm. According to our last, very tentative, version of

the nerve analog the assumed volume changes would be confined within the visco-elastic membrane structures—a "skin." Recent electron-microscopical pictures, for instance by Villegas and Villegas (17), seem to indicate that the membrane layer of squid axons has a somewhat spongy structure. It might perhaps be possible that this structure can be subject to swelling or shrinkage, in somewhat the sense we have dared to suggest here.

A final summary of the working mechanism of an excitable electrohydraulic

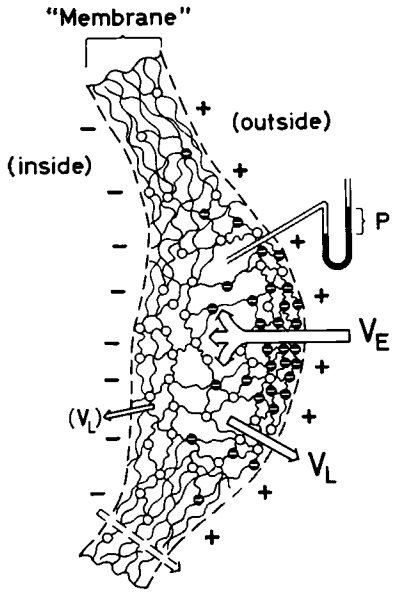

FIGURE 18 A hypothetical excitability unit localised to a negatively charged, elastic membrane structure.

"micro-unit" is given in the figure 19. We see here schematically the two categories of pores, the charged and the leaky one. They are here placed side by side, but they can also be thought of as being concentric layers in the same pore. In the picture the electrical events arising from the resistance variations have been placed entirely within the charged pore. We should also notice perhaps the most controversial item in this theory, namely the existence of a steady current source (i). The creation of

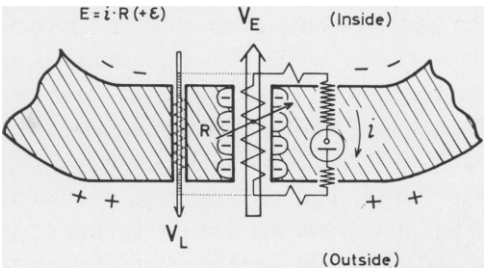

FIGURE 19 A summarising diagram of an electrohydraulic excitable unit.

the resting potential rests here on the assumption of a *resting current.* In turn, this resting current necessitates a kind of a battery. However, this battery must be conceived of as a symbol—simply meaning that a current source driven by the biochemical metabolism is localised somewhere adjacent to the biophysically operating pores. After all, the crucial problem in any of the present theories of nerve action is to explain how the chemical events are coupled to *current formation*—that is *ionic transport.*

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